Repetitive Segmental Structure of the Transducin ß Subunit: Homology with the CDC4 Gene and Identification of Related mRNAs

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Repetitive segmental structure of the transducin β subunit: Homology with the *CDC4* gene and identification of related mRNAs

(guanine nucleotide regulatory protein/photoreceptor biochemistry/molecular evolution/signal transduction)

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Contributed by Melvin I. Simon, November 19, 1985

ABSTRACT **Retinal transducin, a guanine nucleotide regulatory protein (referred to as a G protein) that activates a cGMP phosphodiesterase in photoreceptor cells, is comprised of three subunits. We have identified and analyzed cDNA clones of the bovine transducin β subunit that may be highly conserved or identical to that in other G proteins. From the cDNA nucleotide sequence of the entire coding region, the primary structure of a 340-amino acid protein was deduced. The encoded β subunit has a M_r of 37,375 and is comprised of repetitive homologous segments arranged in tandem. Furthermore, significant homology in primary structure and segmental sequence exists between the β subunit and the yeast CDC4 gene product. The M_r 37,375 β subunit polypeptide is encoded by a 2.9-kilobase (kb) mRNA. However, there exists in retina other β-related mRNAs that are divergent from the 2.9-kb mRNA on the basis of oligonucleotide and primer-extended probe hybridizations. All mammalian tissues and clonal cell lines that have been examined contain at least two β-related mRNAs, usually 1.8 and 2.9 kb in length. These results suggest that the mRNAs are the processed products of a small number of closely related genes or of a single highly complex β gene.**

The transduction of sensory signals involves a family of guanine nucleotide-binding proteins, referred to as N or G proteins (1, 2). Different G proteins are known to mediate the stimulation or inhibition of target biochemical processes in response to activation of specific receptors by light, hormones, neurotransmitters, and other chemical signals. Retinal transducin is a well-characterized G protein that activates a cGMP phosphodiesterase in photoreceptor rod cells and, like other G proteins, it is comprised of three subunits (3). The transducin α subunit (T_α) contains the guanine nucleotide-binding site and reversibly associates with a complex of the β and γ subunits (T_β and T_γ), two tightly bound polypeptides with reported M_rs of about 36,000 and 8400, respectively.

The structure and function of transducin has been studied by the analysis of cDNA clones for the γ and α subunits (4–10). The molecular cloning of cDNA for the α subunit of bovine transducin has revealed the presence of two closely related retina-specific proteins that possess significant sequence homology to each other, *ras* oncogene proteins and elongation factors of protein synthesis. T_γ and Ras proteins were also noted to have similar COOH-terminal sequences (4).

The expression of T_γ and T_α gene products appears to be retina specific (9, 10). On the other hand, the β subunits of transducin and of other G proteins from various tissues are structurally highly conserved on the basis of immunological studies, amino acid composition, and proteolytic peptide mapping, suggesting that the β gene product may be ubiquitous (11–14). In addition, functional similarities between transducin β and the β subunit of other G proteins have been reported (15–17). However, two forms of the β polypeptide with M_rs of about 36,000 and 35,000 have been observed (18). To further define the structure and function of the β subunit we have isolated corresponding cDNA clones. In this paper we report and analyze the complete amino acid sequence of the β subunit and characterize the expression of β subunit mRNA in various tissues.

MATERIALS AND METHODS

Purification of T_β and Protein Sequence Analysis. Bovine transducin was isolated from rod outer segment membranes as described (19), and $T_{\beta\gamma}$ was purified by hydrophobic interaction chromatography on heptylagarose. It was partially cleaved with trypsin into fragments with M_rs of 27,000 and 14,000 and the purified M_r 27,000 tryptic peptide fragment of T_β was subjected to automated Edman degradation (20).

Preparation of Oligodeoxynucleotides, Isolation of cDNA, and DNA Sequence Analysis. Oligodeoxynucleotides were synthesized on an automated DNA synthesizer using phosphoramidite chemistry (21). The bovine retinal cDNA library in the λgt10 phage vector was generously provided to us by Jeremy Nathans (22) and screened with mixed oligonucleotide probes as described (4). DNA sequencing was performed by the dideoxynucleotide chain-termination method (23). Synthetic oligonucleotides, 21 bases in length, were used as sequencing primers.

Sequence Searches and Comparisons. The bovine T_β sequence was searched against an updated version of the protein sequence collection known as NEWAT (24). The sequences and various segments of given proteins were subjected to a rigorous set of alignment and comparison procedures designed to assess the significance of relationships between them (25). In particular, alignments were made with an algorithm similar to that of Needleman and Wunsch (26) in concert with the weighted scoring system derived from the Dayhoff log-odds mutation matrix (27). Dot-matrix plots depicting similar segments were obtained with a program written by Mark S. Johnson.

Primer-Extended Hybridization Probe Synthesis. A cDNA probe was constructed by primer-extension (28) using universal primers and M13 DNA templates that contained the 1386-base-pair (bp) *Eco*RI fragment from the λTB112 cDNA clone.

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Abbreviations: T_α, T_β, and T_γ, α, β, and γ subunits of transducin; bp, base pair(s); kb, kilobase(s); G protein, a family of guanine nucleotide-binding proteins.

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RNA Blot Hybridization Analysis. Total poly(A)$^+$ RNA from quick-frozen bovine and mouse tissues was prepared essentially as described (29). RNA samples were size-fractionated by formaldehyde/agarose gel electrophoresis (30) and transferred directly to nitrocellulose or nylon membranes (31).

Hybridizations of primer-extended or nick-translated probes to RNA fixed on nitrocellulose or nylon blots were performed in 50% formamide/0.75 M NaCl/0.075 M sodium citrate/50 mM NaH_2PO_4, pH 7.0/2× concentrated Denhardt's solution/50 μg of salmon sperm DNA per ml/0.1% $NaDodSO_4$ at 42°C or 35°C as indicated (Denhardt's solution is 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone). The filters were washed at room temperature four times for 5 min in 0.3 M NaCl/0.03 M sodium citrate/0.1% $NaDodSO_4$ and washed subsequently two times for 15 min in 30 mM NaCl/3 mM sodium citrate/0.1% $NaDodSO_4$ at 50°C or as indicated and exposed at −70°C to Kodak XAR-5 film with an intensifying screen (DuPont Cronex Lightning Plus).

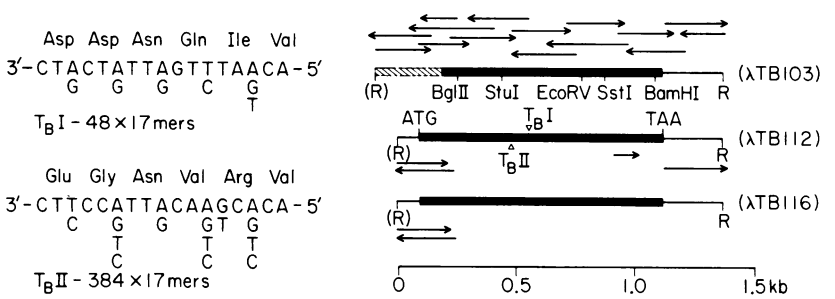
Hybridization of sequencing primer probes to total poly(A)$^+$ RNA from bovine retina was carried out in 6× concentrated SET buffer (0.9 M NaCl/180 mM Tris·HCl, pH 8.0/6 mM EDTA)/10× concentrated Denhardt's solution/0.1% $NaDodSO_4$ at 50°C for 18 hr. The filter blots were successively washed at 0–4°C, 55°C, and 70°C in 0.9 M NaCl/0.09 M sodium citrate/0.1% $NaDodSO_4$ two times for 15 min at each temperature. After each washing condition, the blots were exposed to film for 20 hr.

RESULTS

Partial Amino Acid Sequence of T_β. The T_β was isolated and found to be refractory to peptide sequencing by Edman degradation, suggesting that its NH_2 terminus was "blocked." Limited digestion with trypsin yielded a M_r 27,000 proteolytic fragment. The NH_2-terminal sequence including 29 residues of the purified fragment was determined by Edman degradation. The sequence, amino acids 130–158, is shown in Fig. 1.

Identification and Sequence of cDNA Clones Encoding T_β. About 77,000 plaques of a λgt10 bovine retinal cDNA library were screened with a mixture of 48 different 17-base-long oligonucleotides (T_βI, Fig. 1). Twenty-nine positive clones were isolated and subsequently were screened with another set of nonoverlapping oligonucleotide probes, referred to as T_βII. Probe T_βII consisted of 384 different heptadecamers that were complementary to mRNA sequence corresponding to the first six NH_2-terminal residues of the M_r 27,000 tryptic fragment (Fig. 1). Twenty-one clones were found that hybridized to T_βI and T_βII probes under stringent conditions. DNA was prepared from eight selected clones, digested with *Eco*RI, and analyzed by hybridization to probe T_βII after gel electrophoresis. A single *Eco*RI fragment in each clone hybridized to probe T_βII, and three of these *Eco*RI fragments from the individual phage isolates called λTB103, λTB112, and λTB116 were subcloned into the M13 mp10 phage vector for nucleotide sequence analysis.

A restriction map and the overlapping nucleotide sequence of the cloned *Eco*RI inserts are shown in Fig. 1. The sequence

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GGCTCTGGGG GACTGAGGAC AGTGAGGAGA GCAACAGAGA CGTCACATAT CTCAGATTCT TTGAGGTGGC TTTGAAGAGC ACTAAGATTA GAAG   94
ATG AGT GAA CTT GAC CAG TTA CGG CAG GAG GCC GAA CAG CTT AAA AAC CAA ATT AGA GAT GCT CGG AAG GCG TGT GCA GAT  175
  1 Met Ser Glu Leu Asp Gln Leu Arg Gln Glu Ala Glu Gln Leu Lys Asn Gln Ile Arg Asp Ala Arg Lys Ala Cys Ala Asp
                                                                                  <------------16
GCG ACT CTC TCT CAG ATC ACA AAC AAC ATC GAC CCT GTG GGA AGA ATC CAG ATG CGC ACC AGG AGG ACG CTG AGG GGA CAC  256
 28 Ala Thr Leu Ser Gln Ile Thr Asn Asn Ile Asp Pro Val Gly Arg Ile Gln Met Arg Thr Arg Arg Thr Leu Arg Gly His

TTG GCC AAG ATC TAT GCC ATG CAC TGG GGC ACC GAC TCC AGG CTT CTA GTC AGT GCC TCG CAG GAT GGT AAA CTT ATT ATC  337
 55 Leu Ala Lys Ile Tyr Ala Met His Trp Gly Thr Asp Ser Arg Leu Leu Val Ser Ala Ser Gln Asp Gly Lys Leu Ile Ile
                                                                  <----------14
TGG GAC AGC TAC ACC ACA AAC AAG GTC CAT GCC ATC CCT CTG CGC TCC TCG TGG GTC ATG ACG TGC GCT TAT GCC CCT TCT  418
 82 Trp Asp Ser Tyr Thr Thr Asn Lys Val His Ala Ile Pro Leu Arg Ser Ser Trp Val Met Thr Cys Ala Tyr Ala Pro Ser

GGG AAC TAC GTG GCC TGT GGA GGC CTG GAT AAC ATC TGC TCC ATT TAC AAT CTG AAA ACT CGT GAA GGG AAT GTA CGT GTG  499
109 Gly Asn Tyr Val Ala Cys Gly Gly Leu Asp Asn Ile Cys Ser Ile Tyr Asn Leu Lys Thr Arg Glu Gly Asn Val Arg Val
                                                  <----------12
AGT CGT GAG CTG GCT GGA CAC ACA GGT TAC CTG TCC TGC TGC CGT TTT CTG GAT GAC AAT CAG ATT GTT ACC AGC TCT GGA  580
136 Ser Arg Glu Leu Ala Gly His Thr Gly Tyr Leu Ser Cys Cys Arg Phe Leu Asp Asp Asn Gln Ile Val Thr Ser Ser Gly

GAC ACC ACC TGT GCC CTG TGG GAC ATC GAG ACT GGC CAG CAG ACG ACC ACA TTC ACT GGA CAC ACC GGA GAC GTC ATG AGC  661
163 Asp Thr Thr Cys Ala Leu Trp Asp Ile Glu Thr Gly Gln Gln Thr Thr Thr Phe Thr Gly His Thr Gly Asp Val Met Ser

CTT TCA CTC GCT CCG GAC ACC AGA CTG TTT GTC TCT GGT GCT TGT GAC GCT TCA GCC AAA CTC TGG GAC GTG CGA GAA GGG  742
190 Leu Ser Leu Ala Pro Asp Thr Arg Leu Phe Val Ser Gly Ala Cys Asp Ala Ser Ala Lys Leu Trp Asp Val Arg Glu Gly
                                                                                  <----------6
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217 Met Cys Arg Gln Thr Phe Thr Gly His Glu Ser Asp Ile Asn Ala Ile Cys Phe Phe Pro Asn Gly Asn Ala Phe Ala Thr

GGC TCA GAC GAT GCC ACC TGC CGG CTG TTC GAC CTC CGT GCG GAC CAG GAG CTC ATG ACC TAC TCC CAT GAC AAC ATC ATC  904
244 Gly Ser Asp Asp Ala Thr Cys Arg Leu Phe Asp Leu Arg Ala Asp Gln Glu Leu Met Thr Tyr Ser His Asp Asn Ile Ile
                                                                  <----------4
TGC GGG ATC ACC TCC GTC TCC TTC TCC AAG AGC GGG CGC CTC CTT CTC GCA GGG TAC GAC GAC TTC AAC TGC AAC GTC TGG  985
271 Cys Gly Ile Thr Ser Val Ser Phe Ser Lys Ser Gly Arg Leu Leu Leu Ala Gly Tyr Asp Asp Phe Asn Cys Asn Val Trp

GAC GCC CTC AAG GCC GAC AGG GCA GGG GTC TTG GCC GGG CAT GAC AAC CGC GTG AGC TGC CTG GGG GTA ACT GAT GAC GGG 1066
298 Asp Ala Leu Lys Ala Asp Arg Ala Gly Val Leu Ala Gly His Asp Asn Arg Val Ser Cys Leu Gly Val Thr Asp Asp Gly
                                                                                  7------>
ATG GCC GTG GCG ACG GGA TCC TGG GAC AGC TTC CTC AAA ATC TGG AAC TAACCC TGACAGCAGG TGGACGCCAC AGTGACTGGA AGA 1153
325 Met Ala Val Ala Thr Gly Ser Trp Asp Ser Phe Leu Lys Ile Trp Asn ---
                                                  <----------2
CCATTCC AGCCTTGGAG CGTTGCAGAT AGCATTTCCC ATCTACCCCT ACTAACGCGG ACGCCCTGCA CCCCTCAGAA CTTCAAAAGG GCAAGACCTT 1250
CCCCTCACTT ATTGCTGAAA CCAAGAGCAC AATTCCCACT GAGAGAAGAA TCTCTGTGCT GTAACTAAAA CGAATCGTGC ATTCCTTCCG GGACCAT 1347
TGT CTTTGTTTTC TTTTTTTGTT TGTTTTGAAT GAATTC 1386
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FIG. 1. (*Upper*) DNA sequence analysis and oligonucleotide hybridization probes T_βI and T_βII. Three *Eco*RI fragments were isolated from different cDNA clones labeled as λTB103, λTB112, and λTB116. The portion of each cDNA that encodes T_β is indicated by the bold line and the ATG and TAA codons. The hatched area appears to be extraneous DNA that is not part of any retinal mRNA. The restriction map is diagramed on the λTB103 fragment with *Eco*RI sites abbreviated as R and *Eco*RI linkers enclosed in parentheses. DNA regions that were sequenced are described by the arrows, and loci to which the oligonucleotide probes T_βI and T_βII hybridize are shown by the triangles. kb, Kilobases. (*Lower*) Nucleotide and amino acid sequence derived from T_β subunit cDNA clones. Partial amino acid sequence directly determined by Edman degradation of a M_r 27,000 tryptic peptide fragment of T_β is underlined. Numbering of the nucleotide sequence is on the right and amino acid sequence is on the left. Antisense- (2, 4, 6, 12, 14, and 16) and sense-strand (7) oligonucleotides, 21 bases in length, are symbolized with arrows over their corresponding nucleotide sequence and were used as sequencing primers and hybridization probes.

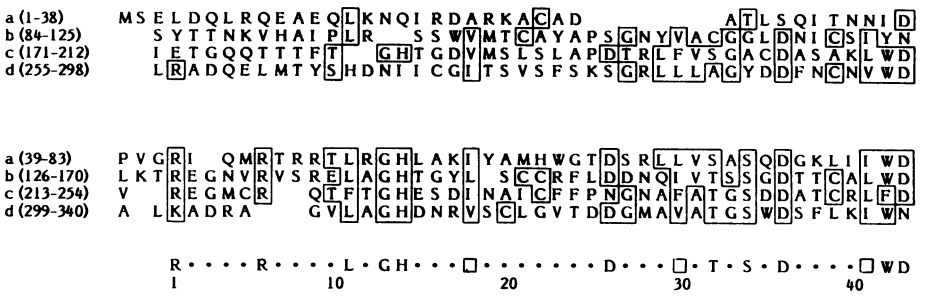

FIG. 2. Homologous segments of the β subunit. The amino acid sequence of the β subunit was aligned as ≈43-residue or ≈86-residue (segments a–d) repeat units by using computer programs described previously (25, 26). Identical or highly conserved residues that align more than three times in the homologous segments are boxed. A consensus sequence including some of the most highly conserved residues is presented below the aligned sequence; hydrophobic residues are symbolized with a square. Numbering on the left indicates the amino acid positions.

of the 1.5-kb *Eco*RI fragment from isolate λTB103 was determined by using synthetic oligonucleotide primers. The sequence of the first 185 nucleotides at the 5′ end of the cDNA was obtained from two independent T_β cDNA clones, λTB112 and λTB116. The 5′ sequences of the cDNA inserts in λTB112 and λTB116 overlapped the open reading frame found in λTB103, which possessed a different 5′ sequence. The 5′ region of λTB103 is probably an artifact of cloning, since, unlike the 5′ cDNA region of λTB112 and λTB116, it is not present in any of the other T_β cDNA clones and does not hybridize to T_β mRNA in RNA transfer blot analysis (results not shown).

The NH_2-terminal protein sequence of the M_r 27,000 T_β tryptic peptide was found to be precisely encoded within the cDNA sequence and is preceded by a basic residue, arginine, as expected for cleavage by trypsin. Thus, the DNA sequence in Fig. 1 encodes the β subunit of transducin. The open reading frame extends from the first ATG codon at nucleotide 95 until an in-phase termination signal, the TAA codon at nucleotide 1115. The protein is 340 amino acids long, and its M_r is 37,375, which is close to M_r 36,000, the estimated size for the transducin β subunit (3). Further, the amino acid composition agrees closely with the reported amino acid analysis of β subunits (11).

The cDNA sequence in Fig. 1 includes 94 bases of 5′-untranslated DNA but shows only 272 bases of 3′-untranslated DNA. The *Eco*RI site at nucleotide 1381 was found in each independent T_β clone that was examined, and the cDNA sequence in Fig. 1 does not extend to the 3′ terminus of the message.

Primary Structure of the β Subunit. At first glance, the amino acid sequence of the β subunit displayed several identical or highly conserved stretches of amino acids, which appeared to comprise larger segments of repeated homologous sequences. The best alignment by computer analysis resulted in a repeat unit of about 86 residues composed of two ≈43 amino acid segments (Fig. 2). A reiterated pattern of amino acid sequences was clear. A consensus pattern is shown in Fig. 2; residues Gly-13, His-14, Asp-36, Trp-42, and Asp-43 are highly conserved, as are the hydrophobic residues at consensus positions 11, 18, 29, 30, 31, and 41. Other identities and conserved substitutions are seen throughout the aligned sequences.

To look for other proteins that share sequence homology with T_β the NEWAT protein sequence database was searched. The only sequence found to show significant resemblance to T_β was the COOH-terminal portion of the yeast CDC4 gene product. The CDC4 protein is composed of 779 amino acids (32, 33). The full-length segment from amino acid 361 to 718 is shown aligned with the 340 amino acid sequence of T_β (Fig. 3). The authentic alignment score was +4.8 standard deviations above the mean of 64 jumbled comparisons of sequences of the same lengths and compo-

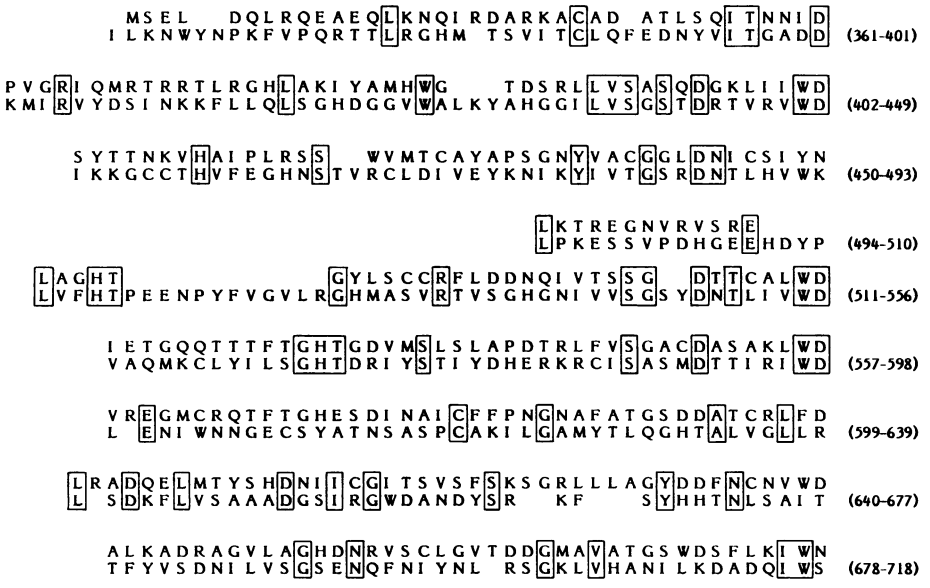

FIG. 3. Homology with yeast CDC4. The alignment of the CDC4 sequence with that of T_β was optimized and the appropriate gaps were introduced by computer analysis. The rows of sequences are arranged according to the segments of the β protein. Amino acid residues that are identical are boxed. Numbering on the right indicates the amino acid positions in CDC4.

sitions. Selected segments within these sequences scored even higher. Although the overall resemblance amounts to only 18–19% identity, there is little doubt that these proteins share common ancestry. The repetitive homologous segments within the CDC4 protein and the β subunit and the homology between these proteins emerge clearly from the dot-matrix analyses shown in Fig. 4.

Expression and Diversity of the β Subunit and Related mRNAs. Hybridization of a primer-extended cDNA probe to poly(A)$^+$ RNA revealed two forms of mRNA, about 2.9 and 1.8 kb in length, in all bovine and mouse tissues that were examined (Fig. 5) as well as in cloned mouse, rat, and human cell lines (data not shown). To determine which of the two mRNA bands represents the T_β cDNA clone that was isolated, the 305-bp *Bam*HI–*Eco*RI restriction fragment, which consisted primarily of 3′-untranslated sequences, was subcloned into the pUC-9 vector, labeled by nick-translation, and hybridized to retinal poly(A)$^+$ RNA. In contrast to previous results using the full-length probe, only one major mRNA with the size of 2.9 kb hybridized to the 3′-region plasmid probe (Fig. 6, lane R).

To further characterize the different mRNAs, a series of sequencing primer probes was hybridized to replicate filter blots of size-fractionated retinal poly(A)$^+$ RNA (Fig. 6, lanes B–Q). The probes hybridize throughout the length of the reported sequence to the small regions that are indicated in Fig. 1. The filters were washed successively at increasing temperature: first, 0–4°C, 55°C, and, then, 70°C. Only one mRNA hybridized to all T_β-specific sequencing primers after stringent washing, and, therefore, this mRNA, about 2.9 kb in length, corresponds to the cDNA clone that was isolated. However, at least two other mRNA species, about 4.3 and 1.8 kb in length, also hybridized to a number of the probes. These same mRNA species were seen when a full-length hybridization probe was used (Fig. 6, lane A). The hybridizations occurred in a specific manner since only the primer probes 6, 12, 14, and 16 hybridized to the additional 4.3- and 1.8-kb mRNAs, whereas the 3′-specific probes 2 and 4 hybridized only to the 2.9-kb mRNA (results for primer 4 not shown). A control probe, 18, showed no hybridization to any of the mRNAs or to the relatively abundant 28S and 18S rRNAs. As the washing temperature was increased to 55°C, the oligonucleotide probes were first melted from the 1.8-kb transcript and washing at 70°C removed most of the remaining probe from the 4.3-kb mRNA. However, the entire series of probes remained hybridized to the 2.9-kb mRNA.

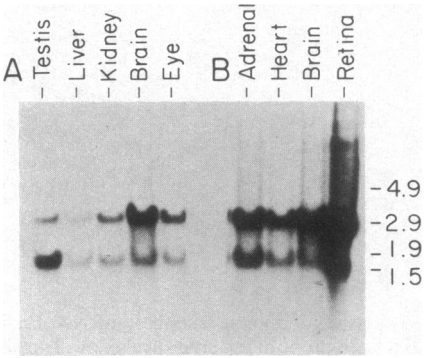

FIG. 5. β Subunit mRNA in bovine and mouse tissues. (*A*) Each lane contained 2 μg of total poly(A)$^+$ RNA from a mouse tissue. (*B*) Each lane contained 8 μg of total poly(A)$^+$ RNA from bovine tissue as indicated. A primer-extended probe was hybridized to the RNA blots at 42°C and washed at 50°C. At the right are indicated the positions and sizes of 28S, 23S, 18S, and 16S rRNA standards (in kilobases).

DISCUSSION

It is apparent that the β polypeptide consists of contiguous homologous segments, each about 43 amino acid residues in length. The consensus motif in the repeated segments of the β protein include the less frequently found amino acid residues tryptophan and histidine at periodic intervals. Furthermore, the relative positions of specific cysteines and aspartate residues are maintained in each segment. The conserved pattern suggests that the protein structure has evolved by duplication and divergence from a basic ≈43 amino acid sequence. Furthermore, the conserved features might be involved in a specific function of the protein, such as ligand or metal ion binding or an enzymatic activity. Repetitive homologous sequences have been found in a number of proteins, including the epidermal growth factor precursor (34–36) and the ribosomal S1 protein (37). Recently, the *Xenopus* 5S rRNA transcription factor TFIIIA, a zinc-binding protein (38), was noted to have a repeating segmented structure (39). The repeated segments are thought to form zinc-binding and DNA–interaction sites. The specific function of the conserved amino acids in the β subunit is not yet apparent.

The primary structure and segmental sequence of the β subunit bear striking resemblance with a portion of the yeast *CDC4* gene. It is quite evident that *CDC4* also has multiple copies of the conserved amino acid sequence motif that is found in the segments of the β subunit. In *CDC4* the consensus motif also appears to repeat periodically, indicating contiguous segments equal in size or slightly larger than the ≈43-residue-long segments of the β subunit. The higher-order homologies such as the conserved consensus motif, the periodic repetition of the motif, as well as the overall sequence homology argue that the two proteins descended from a common ancestor. The β subunit and the CDC4

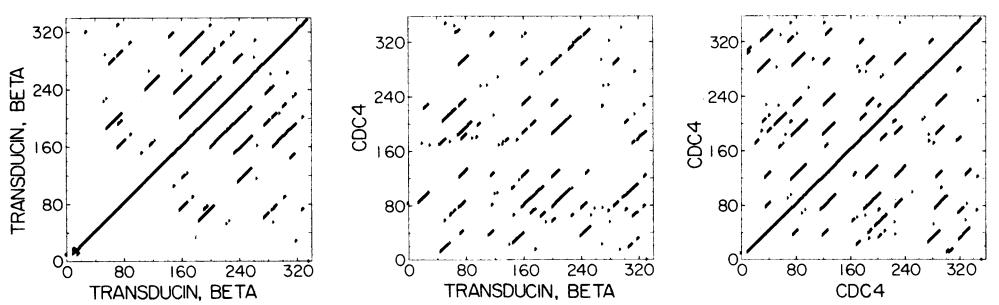

FIG. 4. Dot-matrix plots of bovine transducin β subunit sequence vs. itself (*Left*), transducin β subunit seq ce vs. residue 361–718 of yeast CDC4 (*Center*), and the same region of yeast CDC4 vs. itself (*Right*). The analyses were performed with a moving 20-residue window (segment size = 20) and employed the weighted scoring system derived from the Dayhoff log-odds mutation matrix (27). The cut-off score for plotting a segment was 180 in each case. Note that the diagonal lines denoting similar segments occur at approximately 40- to 45-residue intervals in all three cases.

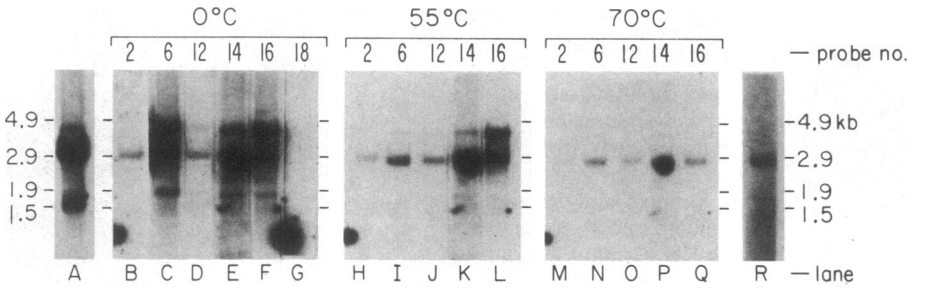

FIG. 6. Expression of diverse β subunit mRNA. Each lane contained 1 μg of total poly(A)$^+$ RNA from bovine retina. Lane A, hybridization to a primer-extended probe at lowered stringency. The blot was hybridized at 35°C and washed at 40°C in 0.3 M NaCl/0.03 M sodium citrate/0.1% $NaDodSO_4$ two times for 15 min. Lane R, hybridization to a nick-translated plasmid probe containing 305 bp of 3'-region cDNA. The filter was hybridized at 42°C and washed up to 52°C. Lanes B–Q, hybridization as indicated to sequencing primer probes 2, 6, 12, 14, 16, and 18. Oligonucleotide 18 hybridizes within the hatched region of the λTB103 insert and was used as a control (see Fig. 1 *Upper*). Hybridization was performed at 50°C and washing was carried out successively at 0°C (lanes B–G), 55°C (lanes H–L), and 70°C (lanes M–Q). RNA size markers are indicated on the left and right.

protein may also share similar functions. *CDC4* is a yeast cell-division-cycle gene that is required in an early event in the mitotic cycle (40, 41). A primary activity of the β subunit is its interaction with the GTP-binding α subunits. In yeast the Ras proteins, which have regions of amino acid homology with α subunits (42), are thought to play a role analogous to that of the α subunit of the G_s protein—i.e., they activate adenylate cyclase in a GTP-dependent mechanism (43). Perhaps, just as α interacts with the β subunit in G proteins, Ras may interact with the CDC4 gene product as part of an information-processing system required to integrate metabolic events with the yeast cell cycle. On the other hand, the β subunit may have a role in the control of the cell cycle in addition to its function as part of the G protein complex.

Our results also indicate that there is a diversity of β subunit-related mRNAs that could encode different proteins. It seems likely that there are a number of highly homologous β-related genes on the basis of hybridization with genomic DNA (results not shown); however, we cannot exclude the possibility that multiple cross-hybridizing messages arise from a single highly complex gene. Identification of β-related gene products and investigation of their role in the mechanisms involved in signal transduction and the regulation of cell growth await further study.

We express sincere thanks to Drs. Breck Byers and John Yochem for communicating to us the sequence of the yeast *CDC4* gene and Drs. Michael A. Lochrie for many helpful discussions and ideas, Jeremy Nathans for providing us with the bovine retinal cDNA library, Suzanna Horvath for synthesizing the oligodeoxynucleotides, David B. Teplow for assistance with the peptide sequence analysis, and Eric Phizicky for providing heptylagarose. This work was supported by Grant GM34236 from the National Institutes of Health.

1. Rodbell, M. (1980) *Nature (London)* **284,** 17.
2. Gilman, A. G. (1984) *Cell* **36,** 577–579.
3. Stryer, L. (1985) *Biopolymers* **24,** 29–47.
4. Hurley, J. B., Fong, H. K. W., Teplow, D. B., Dreyer, W. J. & Simon, M. I. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6948–6952.
5. Yatsunami, K., Pandya, B. V., Oprian, D. D. & Khorana, H. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1936–1940.
6. Lochrie, M. A., Hurley, J. B. & Simon, M. I. (1985) *Science* **228,** 96–99.
7. Tanabe, T., Nukado, T., Nishikawa, Y., Sugimoto, K., Suzuki, H., Takahashi, H., Noda, M., Haga, T., Ichiyama, A., Kangawa, K., Minamino, N., Matsuo, H. & Numa, S. (1985) *Nature (London)* **315,** 242–245.
8. Yatsunami, K. & Khorana, H. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4316–4320.
9. Medynski, D. C., Sullivan, K., Smith, D., Van Dop, C., Chang, F., Fung, B. K.-K., Seeburg, P. H. & Bourne, H. R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4311–4315.
10. Van Dop, C., Medynski, D., Sullivan, K., Wu, A. M., Fung, B. K.-K. & Bourne, H. R. (1984) *Biochem. Biophys. Res. Commun.* **124,** 250–255.
11. Manning, D. R. & Gilman, A. G. (1983) *J. Biol. Chem.* **258,** 7059–7063.
12. Audiger, Y., Pantaloni, C., Bigay, J., Deterre, P., Bockaert, J. & Homburger, V. (1985) *FEBS Lett.* **189,** 1–7.
13. Gierschik, P., Codina, J., Simons, C., Birnbaumer, L. & Spiegel, A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 727–731.
14. Huff, R. M., Axton, J. M. & Neer, E. J. (1985) *J. Biol. Chem.* **260,** 10864–10871.
15. Kanaho, Y., Tsai, S.-C., Adamik, R., Hewlett, E. L., Moss, J. & Vaughan, M. (1984) *J. Biol. Chem.* **259,** 7378–7381.
16. Northrup, J. K., Sternweis, P. C. & Gilman, A. G. (1983) *J. Biol. Chem.* **258,** 11361–11368.
17. Bockaert, J., Deterre, P., Pfister, C., Guillon, G. & Chabre, M. (1985) *EMBO J.* **4,** 1413–1417.
18. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259,** 13806–13813.
19. Fung, B. K.-K., Hurley, J. B. & Stryer, L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 152–156.
20. Hunkapiller, M. W. & Hood, L. E. (1983) *Methods Enzymol.* **91,** 486–493.
21. Hunkapiller, M., Kent, S., Caruthers, M., Dreyer, W., Firca, J., Giffin, C., Horvath, S., Hunkapiller, T., Tempst, P. & Hood, L. (1984) *Nature (London)* **310,** 105–111.
22. Nathans, J. & Hogness, D. S. (1983) *Cell* **34,** 807–814.
23. Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
24. Doolittle, R. F. (1981) *Science* **214,** 149–159.
25. Feng, D. F., Johnson, M. S. & Doolittle, R. F. (1985) *J. Mol. Evol.* **21,** 112–125.
26. Needleman, S. B. & Wunsch, C. D. (1970) *J. Mol. Biol.* **48,** 443–453.
27. Dayhoff, M. O., ed. (1978) *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Washington, DC), Vol. 5, Suppl. 3.
28. Hu, N.-T. & Messing, J. (1982) *Gene* **17,** 271–277.
29. Drubin, D. G., Caput, D. & Kirschner, M. W. (1984) *J. Cell Biol.* **98,** 1090–1097.
30. Lehrach, H., Diamond, D., Wozney, J. M. & Broedtker, H. (1977) *Biochemistry* **16,** 4743.
31. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
32. Peterson, T. A., Yochem, J., Byers, B., Nunn, M. F., Duesberg, P. H., Doolittle, R. F. & Reed, S. I. (1984) *Nature (London)* **309,** 556–558.
33. Yochem, J. (1985) Dissertation (University of Washington, Seattle, WA).
34. Gray, A., Dull, T. J. & Ullrich, A. (1983) *Nature (London)* **303,** 722–725.
35. Scott, J., Urdea, M., Quiroga, M., Sanchez-Pescador, R., Fong, N., Selby, M., Rutter, W. J. & Bell, G. I. (1983) *Science* **221,** 236–240.
36. Doolittle, R. F., Feng, D. F. & Johnson, M. S. (1984) *Nature (London)* **307,** 558–560.
37. Doolittle, R. F., Woodbury, N. W. & Jue, R. A. (1982) *Biosci. Rep.* **2,** 405–412.
38. Hanas, J. S., Hazuda, D. J., Bogenhagen, D. F., Wu, F. Y. H. & Wu, C. W. (1983) *J. Biol. Chem.* **258,** 14120–14125.
39. Miller, J., McLachlan, A. D. & Klug, A. (1985) *EMBO J.* **4,** 1609–1614.
40. Byers, B. & Goetsch, L. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **38,** 123–131.
41. Hartwell, L. H. (1971) *J. Mol. Biol.* **59,** 183–194.
42. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D. & Gilman, A. G. (1984) *Science* **226,** 860–862.
43. Toda, T., Uno, I., Broach, J., Matsumoto, K. & Wigler, M. (1985) *Cell* **40,** 27–36.